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# The mechanisms of marine bacterial interactions

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## Summary

Bacteria are found in numerous habitats within the vast marine realm. Many marine bacteria live together as a biofilm which allows them to closely interact and intimately influence each other's lives, mainly by the production of extracellular compounds such as antibiotics and exopolysaccharides. Coordination of the biofilm and communication between bacteria is controlled by a mechanism known as quorum sensing in which signal molecules called autoinducers are released and detected, resulting in an alteration of behaviour. These activities ultimately have implications on other organisms; controlling populations of invertebrates and algae, and affecting the health of eukaryotes, a major cause for concern in coral reefs worldwide. This paper reviews the interactions amongst bacterial species and the mechanisms directing them. It briefly discusses their effect on eukaryotes and hints at the increasing importance of understanding interactions between coral associated bacteria, and the mechanisms of bacterial infection in corals.

## 1. Introduction

Bacteria inhabit a wide range of habitats in the world's oceans (Table 1). In these habitats, they often live closely with other microorganisms such as archaea, microalgae and viruses; and it has been estimated that a total number of $1.18 \times 10^{29}$ prokaryotes live in the oceans (Whitman *et al.* 1998) with $3.1 \times 10^{28}$ of these being bacterial cells (Karner *et al.* 2001).

As with the high number of bacterial habitats, there are also a high number of bacterial interactions. These can be intra- and inter-specific within the bacteria, where they can communicate using novel communication systems, and can kill or affect the growth of other species by production and release of bioactive compounds. Bacterial interactions also often affect the behaviour or health of higher organisms. Ultimately, marine bacterial interactions and activities affect a high number of marine processes, such as the microbial loop and algal blooms (Munn 2004).

**Table 1** Bacterial habitats in the marine environment

| **Habitat** | **Notes** |
|---|---|
| Pelagic | - Proteobacteria (particularly α- and γ- Proteobacteria) and Bacteroidetes are the most abundant heterotrophs (Stevens *et al.* 2005).<br>- Free-living bacteria show a higher diversity and lower abundance than bacteria attached to particles, such as marine snow (Acinas *et al.* 1997).<br>- The most predominant bacteria near surface waters is SAR11, an α-Proteobacterium (Acinas *et al.* 1999) which has also been found to be the most abundant bacteria in the marine environment (Munn 2004). |
| Sediments | - 90% of prokaryotes (including bacteria) can be found in deep subsurface sediments (Munn 2004)<br>- Bacterial communities in deep sediments are very similar to those at cold seeps and vents (Munn 2004). |
| Hydrothermal vents and cold seeps | - Vents are hotspots of microbial activity with a significant decrease in prokaryotic abundance with increasing distance from the vent (Manini *et al.* 2008).<br>- They contain large populations of chemolithotrophic bacteria (Munn 2004).<br>- Cold seeps contain high concentrations of free-living and symbiotic chemosynthetic bacteria (Munn 2004). |
| Deep-sea | - The most abundant and genetically diverse bacteria found here are the γ-Proteobacteria (Lopez-Garcia *et al.* 2001). |
| Substrates | - Bacteria create complex biofilms on substrates, which can include plants, rocks and animals (Munn 2004).<br>- The majority of marine bacteria are organised as biofilms (Burmolle *et al.* 2006). |
| Sea-surface microlayer | - This is the least understood of all the microbial habitats, and the bacterial assemblages found here (the bacterioneuston) are poorly characterised (Agogue *et al.* 2005).<br>- Agogue *et al.* (2005) speculate that the microbial concentration of this layer is a lot more abundant than in the underlying water column. |

## 2. Intra- and inter- species interactions

### *2.1 Antagonistic interactions between bacteria*

Antagonistic bacterial interactions involving antibiotics are found everywhere in the marine environment and are exhibited by a high number of bacterial clades. Free-living, sediment-inhabiting and surface-associated bacteria show these antibacterial characteristics, however some studies have found that the production of antibiotics is more common in surface-associated bacteria living in biofilms than in free-living bacteria (Burgess *et al.* 1998; Long and Azam 2001). This suggests that antibiotics are important in structuring surface-associated communities where space is limited and the ability to inhibit growth

of other bacteria is a major advantage (Long and Azam 2001). These surfaces can range from physical components of the marine environment such as rocks, to coral holobionts, algae and marine particles. The resultant cell death or lysis of the bacteria is very important in the turnover of organic matter in the oceans (Grossart *et al.* 2004). One such example of bacteria using antibiotics is the inhibition of the highly pathogenic *Vibrio cholerae* (Long *et al.* 2005). Attachment of *V.cholerae* to marine particles is important in its transmission from water to humans, but Long *et al.* (2005) found that marine bacteria inhabiting these particles produce the antibacterial compound andrimid, which inhibits the proliferation of this pathogen. Many members of the *Roseobacter* clade also produce antibiotics; however their action is primarily against non-*Roseobacter* species (Bruhn *et al.* 2007). Similarly, bacteria associated with sponges and soft corals produce antibacterial compounds active against only planktonic bacteria, not other surface-associated bacteria (Reshef *et al.* 2006). Pigmented members of the *Pseudoalteromonas* genus are also major antibiotic producing bacteria (Bowman 2007) which produce a range of extracellular products from brominated antibiotic compounds to tambjamines and korormycin, acting on a variety of marine bacteria, especially *Vibrio* spp. (Bowman 2007).

A rare use of antibacterial compounds is seen in the genus *Pseudoalteromonas* which exhibits the phenomenon of auto-inhibition and has been observed in at least six species (Holmström and Kjelleberg 1999; Holmström *et al.* 2002; Bowman 2007). The antibacterial compound produced by one of these, *Pseudoalteromonas tunicata* strain D2, has been identified as AlpP (Bowman 2007) and is extremely potent against the strain itself. However, Bowman (2007) found that as *P.tunicata* strain D2 reaches the stationary growth phase, the cells become more resistant to AlpP. Possible explanations for auto-inhibition have been described as to provide nutrients for resistant subpopulations (such as those that have reached the stationary growth phase), to aid in dispersal and colonisation of new substrates (Bowman 2007), and also to maintain microbial diversity within a community (Holmström and Kjelleberg 1999).

### *2.2 Cooperative interactions between bacteria*

Ruby (1996) states that several decades ago, most studies of microbial interactions concentrated on those with detrimental effects, even though cooperative interactions exist.

Many marine bacteria produce high molecular weight (MW) carbohydrate polymers (Kumar *et al.* 2007) called exopolysaccharides (EPS). EPS enhance the survival of the producing bacteria and other organisms living in its vicinity (Holmström and Kjelleberg 1999) by influencing the physiochemical environment around the bacterial cell (Nichols *et al.* 2005). EPS may be particularly important in harsh environments such as salinity and temperature extremes (Guezennec 2002; Nichols *et al.* 2005). Even though many studies (Weiner 1997; Colliec-Jouault *et al.* 2004; Saravanan and Jayachandran 2008) suggest the possible applications and importance of EPS in pharmaceuticals and various industries, Kumar *et al.* (2007) note that the ecological significance of EPS have not yet been realised.

### *2.3 Quorum sensing*

Bacteria are capable of inter- and intra- specific interactions at certain population densities, through a mechanism known as quorum sensing (QS) (Mohamed *et al.* 2008). Simply put, the bacterial QS system consists of the release of chemical signal molecules which, when detected at critical threshold concentrations, lead to changes in gene expression (Miller and Bassler 2001) to allow a competitive advantage (Jayaraman and Wood 2008). The signal molecules are termed autoinducers and are described by Dunn and Stabb (2007) as "bacterial pheromones". QS facilitates a wide range of functions in bacteria such as behaviour alteration, cell-cell communication and in conflicts within and between species (Keller and Surette 2006), influencing settlement and subsequent biofilm formation (Bowman 2007), regulation of gene expression (Fuqua and Greenberg 2002), motility, sporulation, symbiosis and virulence (Miller and Bassler 2001). In their 2002 paper, Fuqua and Greenberg describe the study of bacterial cell-cell communication as a new area in microbiology, and in those early days QS was believed to be an isolated phenomenon found in only a few species (Dunn and Stabb 2007). However, with the increase in studies on this area, it is now thought that QS is extensively used by bacteria and with the use of molecular genomic tools it is coming to light that many of the systems involved in QS are more complex than previously thought (Dunn and Stabb 2007).

It is now known that the autoinducer2 (AI-2) QS pathway produced by LuxS is the only form of QS which is shared by both Gram-positive and Gram-negative bacteria (Hardie and Heurlier 2008; Li *et al.* 2008). However, both adopt their own form of autoinducer in their cell-to-cell communication; Gram-positive bacteria utilise oligo-peptides to communicate (Miller and Bassler 2001), whilst *N*-acyl homoserine lactones (AHLs) are widely used by Gram-negative bacteria (Steindler *et al.* 2008). Mohamed *et al.* (2008) note that intense study has been carried out on AHL signalling in marine vibrios, but limited information can be found on AHL-dependant signalling regulation in other marine bacteria.

Despite the many references above describing the role of QS in bacteria, a paper by Keller and Surette (2006) brings to light how these roles have been challenged by the suggestion that autoinducers are used simply by individual cells to obtain information about the mixing and diffusive properties of their surrounding environment. Although evidence has been found for both cases, this could suggest that QS evolved for other reasons and indeed some microbiologists believe that QS evolved only to allow bacteria to coordinate the population as a whole and act as a multicellular organism (Keller and Surette 2006; Waters and Bassler 2005). Miller and Bassler (2001) suggest just this and go on to postulate that QS systems in bacteria could have been one of the first steps in the evolution of multicellularity.

### *2.4 Effects of bacterial communities on larval settlement*

It is widely known that microbial biofilms influence the settlement of marine larvae and algal spores by producing stimuli for them to respond to (Dahms *et al.* 2004). These stimuli can be an alteration of the surface chemistry and microtopography of a substrate as a result of the biofilm, or it can be more complicated in the release of microbial products from the biofilm (Qian *et al.* 2007).

Bacteria isolated from a surface can be both inhibitory and inductive to the larvae of an invertebrate species. For example, in a study by Dobretsov and Qian (2004) 18% of the bacteria isolated from the surface of the soft coral *Dendronephthya* sp. had inhibitory effects on the settlement of tubeworm *Hydroides elegans* larvae, whilst 36% induced the larvae to settle and the remaining 46% had no effect.

As previously mentioned, the production of compounds by bacteria in the biofilm has a marked effect on larval substrate choices. Much work has been carried out on the mechanisms of this, particularly on members of the genus Pseudoalteromonas which produce natural anti-fouling extracellular compounds (Bowman 2007; Skovhus *et al.* 2007), aiding in the inhibition of settlement in larvae. A link has been made between pigmentation in *Pseudoalteromonas* and production of inhibitory compounds (Egan *et al.* 2002; Holmström *et al.* 2002; Bowman 2007). *Pseudoalteromonas* spp. produce two pigments; yellow and purple (Egan *et al.* 2002). Bowman (2007) identified the yellow pigment as a tambjamine-like alkaloid (YP1). Other anti-fouling compounds have also been identified; those produced by *Vibrio* sp. and an unidentified α-Proteobacterium are heat-stable polysaccharides of MW > 100kDa (Dobretsov and Qian 2004), whilst those produced by *Alteromonas* sp. 1 are heat-stable but much smaller with a MW of < 3kDa (Bao *et al.* 2007). Holmström and Kjelleberg (1999) suggest this anti-fouling ability formed in Pseudoalteromonas, and other bacterial genera, as an aid in the competition for nutrients and space.

It has also been found that AHLs used in bacterial QS can have a positive effect on the settlement of larvae and algal spores (Huang *et al.* 2007; Mohamed *et al.* 2008, Tait *et al.* 2005).

### *2.5 Other effects of bacterial communities on higher organisms*

Aside from larval settlement, interactions within bacterial communities have other implications on the wider environment. The main points of these will be discussed below with further details and examples given in Table 2.

Bacteria can aid in the metamorphosis of settled larvae or spores (Bowman 2007). Mechanisms involved in these interactions are poorly understood, however Holmström and Kjelleberg (1999) explain their observations in larval metamorphosis with bacterial release of EPS, as described in section 2.2. Bacteria can also positively influence the growth of algae once metamorphosis has been completed. Although this particular effect does not appear to be widely studied, it is known that bacteria can cause algae to grow in massive blooms which may lead to major fish kill (Liu *et al.* 2008).

Symbiosis, in which bacteria live in association with animal tissue, is widely occurring and plays an important part in the ecology of many marine bacteria (Ruby 1999). In many cases it is also fundamental to the survival of vertebrates and invertebrates, and can allow an organism to live in a habitat that was previously thought uninhabitable.

Many diseases in marine vertebrates and invertebrates are caused by marine bacteria. This is particularly a problem in aquaculture (Munn 2004) where

stressed individuals are more susceptible to disease, which can spread quickly. Similarly to disease, toxins or "a poison produced by a living organism" (Salzman *et al.* 2006) produced by many marine bacteria cause problems for marine eukaryotes, from severe health threats to death (Holmström and Kjelleberg 1999).

A novel idea was suggested by Negri *et al.* (2001) which involves using bacteria that have positive effects on coral larval settlement and metamorphosis, such as *Pseudoalteromonas* sp. A3, to re-seed reefs with coral species.

**Table 2** Effects of bacterial communities on higher organisms

| **Effect** | **Examples** | **Reference** |
|---|---|---|
| Laval metamorphosis | Bacterial biofilms have been shown to induce invertebrate larvae to metamorphose in greater numbers. | Bowman (2007); Holmström and Kjelleberg (1999) |
| Growth promotion of algae | Bacteria related to the genus *Bacillus* has growth promoting effects on alga species, often causing huge blooms. | Liu *et al.* (2008) |
| Symbiosis | (a) The sepiloid squid *Euprymna scolopes* utilises the luminous bacteria *Vibrio fischeri* in predator avoidance.<br>(b) Over 80 species of gutless, symbiotic oligochaete worms have been discovered, such as *Olavius* spp. which host multiple sulphur-oxidising and sulphate-reducing bacteria, providing nutrition to the worm.<br>(c) There is evidence for the use of QS signals in symbiotic interactions | Ruby (1996); Claes and Dunlap (2000); Dubilier *et al.* (2001); Giere and Erseus (2002); Blazejak *et al.* (2005); Woyke *et al.* (2006); Chun *et al.* (2008); McFall-Ngai (2008) |
| Disease | (a) Diseases in vertebrates include vibriosis in eels and other fish species caused by *Vibrio anguillarum*, mammalian abortion caused by *Brucella,* and tuberculosis caused by *Mycobacterium tuberculosis*.<br>(b) Invertebrate diseases include those in bivalves and crustaceans, often caused by *Vibrio* spp., and those in algae such as red spot disease in *Laminaria japonica,* caused by *Pseudoalteromonas* sp.. A wide range of diseases is found in corals, which is discussed in section 2.3. | Holmström and Kjelleberg (1999); Munn (2004); Nylund *et al.* (2008) |
| Toxins | (a) Pathogenic *Vibrio* spp. produce a variety of toxins harmful to marine vertebrates, marine invertebrates and humans.<br>(b) *Pseudoalteromonas* spp. release toxins such as neuromuscular toxins and the neurotoxin tetrodotoxin.<br>(c) There is evidence that cyanobacterial toxins are responsible for paralytic shellfish poisoning. | Holmström and Kjelleberg (1999); Lueger *et al.* (1999) Zhang and Austin (2005); Bowman (2007); MacQuarrie and Bricelj (2008). |

## 3. Interactions involving coral bacteria

### *3.1 Diversity of coral bacteria*

The coral holobiont is a complex association of the coral animal with endolithic fungi, zooxanthellae, bacteria, archaea and viruses (Wegley *et al.* 2007). Bacterial populations live throughout the different coral components, from the mucus layer to the tissues and the carbonate skeleton. Estimates of bacterial abundance in the mucus layer of corals lie at $10^5$-$10^6$ CFU $ml^{-1}$, with similar estimates for the coral tissue (Rosenberg *et al.* 2007). A high diversity of bacteria exists within the coral animal (Nissimov *et al.* 2009), which can vary greatly between coral species, and even within the different components of the same coral fragment (Rosenberg *et al.* 2007). Rosenberg *et al.* (2007) found the association between a coral and its microbes to be specific, as the bacterial species in the surrounding water were of a different composition to those found within the coral. They also noted that even if corals of the same species are geographically separated, the composition of their bacterial populations remain similar. This specificity indicates that the bacterial populations are carefully assembled to the particular needs or requirements of a coral species, which could be disrupted by environmental change, as will be discussed in the section below.

With regard to groups of bacteria inhabiting the coral, several studies of particular shallow-water corals (Santiago-Vazquez *et al.* 2007; Wegley *et al.* 2007) have found that the prominent group is the proteobacteria (namely γ-proteobacteria and α-proteobacteria) making up 68% of the bacterial population in the coral *Porites asteroides* and 36% in the coral *Cirrhipathes lutkeni*. Following the proteobacteria are the groups *Actinobacteria* (6% and 19% respectively) and *Firmicutes* (10% and 9% respectively). Noting that microbial associations with shallow-water corals were fairly well studied whilst the associations with deep-water corals were left relatively unknown, Penn *et al.* (2006) carried out a study of Alaskan seamount octocoral microflora. They too found that γ-proteobacteria and α-proteobacteria were the prominent bacterial groups. However, many other groups of bacteria are associated with corals giving them their high bacterial diversity, and suggests that the environment of the coral animal allows many species of bacteria to proliferate. Indeed it has been found that coral-associated bacteria grow up to ten times faster than their counterparts in the over-lying water column (Paul *et al.* 1986). The well-known high diversity of bacteria and their genes found within the coral holobiont gives the holobiont potential to adapt quickly to the constantly changing environmental conditions that corals face.

### *3.2 Environmental effects on shifts in coral bacterial communities*

Global warming and the resulting climate change are possibly the most discussed current environmental challenges, emphasised by the sheer number of articles brought up by typing these phrases into the scientific database of journals ISI Web of Science (9,950 and 44,030 respectively [November 2008]). Unsurprisingly, this warming of the environment has great effects on the fragile ecosystems of coral reefs.

It is known that shifts in coral bacterial communities occur with the seasons (Koren and Rosenberg 2006). A periodic discharge of the coral mucus occurs to remove the current bacteria, and new bacteria are acquired from the surrounding seawater, or from previously low populations already existing in the coral (Reshef *et al.* 2006). Animals feeding on the coral, such as the marine fireworm *Hermodice carunculata* (Sussman *et al.* 2003), can also act as a vector for the transfer of bacteria between corals, and as a winter reservoir when water temperatures are too low for the bacteria to survive in the coral.

However, many bacterial shifts occur as a result of elevated seawater temperatures due to global warming. For example, Ritchie (2006) found that during a summer bleaching event *Acropora palmata* tissue had lost antibiotic-producing bacteria and was dominated by pathogenic *Vibrio* spp., which shows a shift *from* beneficial bacteria and questions the consistency of the protective properties of coral mucus. Ritchie *et al.* (1993) also found that a *Vibrio* sp., specifically *V.shiloi*, replaced other bacteria in a bleached coral, this time *Montastrea annularis*. Similarly, the microbial community of *Acropora millepora* shifted during a bleaching event and *Vibrio* spp. were found (Bourne *et al.* 2008). It was also found that in some bleached corals, *Vibrio* populations increased whilst *Pseudoalteromonas* populations decreased, with the opposite effect in healthy or recovering corals (Ritchie and Smith 2003). However, as Rosenberg and Falkovitz (2004) and later Bourne *et al.* (2008) state, in all cases it cannot be certain whether this replacement with *Vibrio* spp. is cause or result of the disease; perhaps it is time that this important question is resolved.
Environmental effects other than temperature also cause shifts in bacterial communities, an example being human contaminants in reef water. One coral, *Diploria strigosa*, showed large shifts in community composition in the presence of contaminants (Klaus *et al.* 2005).

The ultimate shift in coral associated bacteria, however, is illustrated in the Coral Probiotic Hypothesis (CPH) which describes the relationship between the coral host and its associated bacteria that selects for the most advantageous coral holobiont (Reshef *et al.* 2006), with a high proportion of bacteria (probiotics) that give a health benefit to their host (Schrezenmeir and de Vrese 1998). This has implications for the survival of coral reefs (Wegley *et al.* 2007) and is increasingly important in this age of global warming which is allowing bacterial disease in corals to become more prevalent.

### *3.3 Environmental effects on coral disease/bleaching*

Banin *et al.* (2000) note that several studies in 1985 stated that the correlation between seawater temperature and coral bleaching was *not* always evident but, with the expansion of technology in the field such as satellite data, it is now clear that the onset and duration of coral bleaching due to bacterial infection coincides with elevated seawater temperatures (Banin *et al.* 2000; Rosenberg *et al.* 2007). The elevated temperature is a physiological stress for the coral which degrades its health and, among other mechanisms, allows secondary infection by opportunistic bacterial pathogens (Lesser *et al.* 2007) leading to disease and/or bleaching. During the last ten years the number of published studies on coral disease has dramatically increased (Ward and Lafferty 2004) which reflects the increasing importance of studying the interplay of temperature, bacterial infections and corals, as in just the last few decades

about 30% of the world's coral reefs have been intensively damaged by bleaching and disease (Rosenberg and Falkovitz 2004; Rosenberg *et al.* 2007). But contrary to the general belief that increasing seawater temperatures compromise coral resistance to bacterial infection, a study of the seafan *Gorgonia ventilina* (Ward 2007) found that resistance of tissue on the edge of the colony actually increased with temperature stress. Also, although Lesser *et al.* (2007) write that analysis of published work supports the hypothesised increase in prevalence of coral diseases, many recent survey data do not support this. *V.shiloi* has failed to be isolated from healthy and diseased *Oculina patagonica* since 2003 (Rosenberg *et al.* 2007). In the Florida Keys, healthy corals are no longer susceptible to white plague disease and white plague type II (Reshef *et al.* 2006), and the prevalence of coral disease in Mexico is decreasing (Ward *et al.* 2006). These cases could be explained by the CPH, as mentioned in the previous section, although Lesser *et al.* (2007) raise a valid point that the noted decrease in prevalence of bacterial disease could be due mainly to the decrease in susceptible individuals left after the disease has swept through the population.

## 4. Conclusions and implications for research

During this research, I found that much work has been carried out on the mechanisms of interactions of *Pseudoalteromonas spp.* and *V.shiloi* with other bacteria and higher organisms such as coral; for example Ben-Haim *et al.* (1999), Holmström and Kjelleberg (1999), Banin *et al.* (2003), Bowman (2007), Lesser *et al.* (2007), Rosenberg *et al.* (2007) and Skovhus *et al.* (2007), but intriguingly few studies have been conducted on the mechanisms of interactions between the two. I say 'intriguingly' because it is widely known that *V.shiloi* is a major disease-causing pathogen in corals (Rosenberg and Falkovitz 2004) and *Pseudoalteromonas spp.* are known to inhabit corals and readily produce antibacterial compounds active against many bacteria, including *V.shiloi* (Bowman 2007). The studies which *have* been carried out on the interactions between the two bacteria, such as Ritchie and Smith (2003), do not go into depth about the modes or specifics of the interaction and only offer the general pattern in population abundance changes. Thus considering the major role these two bacteria play in defence and attack in relation to disease of coral holobionts, and the great increase in mortality of coral reefs due to disease over the last few decades (Rosenberg and Falkovitz 2004), I feel in-depth studies into this topic would be hugely relevant and important and may even allow the knowledge to implement strategic plans to possibly reduce disease and aid recovery in coral reefs.

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